

09/673476

528 R PCT/PTO 16 OCT 2000

Annexes (amended sheets) to the Preliminary Examination Report

been deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) on XX XX, 1998 under the accession number I-XXXX.

Thus, as a specific embodiment of the above described method for isolating a polynucleotide of interest said method makes use of at least one BAC-based DNA library that has been constructed from the genomic DNA of *Mycobacterium tuberculosis*, more specifically of the H37Rv strain and particularly of the DNA library deposited in the accession number I-1945.

In another specific embodiment of the above described method for isolating a polynucleotide of interest said method makes use of at least one BAC-based DNA library has been constructed from the genomic DNA of *Mycobacterium bovis* BCG, more specifically of the Pasteur strain and particularly of the DNA library deposited in the accession number I-XXXX.

In more details, the method according to the invention for isolating a polynucleotide of interest may comprise the following steps :

- a) isolating at least one polynucleotide contained in a clone of a BAC-based DNA library of mycobacterial origin;
- b) isolating :
 - at least one genomic or cDNA polynucleotide from a mycobacterium, said mycobacterium belonging to a strain different from the strain used to construct the BAC-based DNA library of step a); or alternatively
 - at least one polynucleotide contained in a clone of a BAC-based DNA library prepared from the genome of a mycobacterium that is different from the mycobacterium used to construct the BAC-based DNA library of step a);
- c) hybridizing the at least one polynucleotide of step a) to the at least one polynucleotide of step b);
- d) selecting the at least one polynucleotide of step a) that has not formed a hybrid complex with the at least one polynucleotide of step b);
- e) characterizing the selected polynucleotide.

Following the above procedure, the at least one polynucleotide of step a) may be prepared as follows :

- 1) digesting at least one recombinant BAC clone by an appropriate restriction endonuclease in order to isolate the polynucleotide insert of interest from the vector genetic material;
- 2) optionally amplifying the resulting polynucleotide insert;

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disease and elicits a variable antibody response suggesting either that individuals mount different immune responses or that this PGRS-protein may not be produced in this form by all strains of *M. tuberculosis*. In other words, at least some PE_PGRS coding sequences encode for proteins that are involved in the recognition of *M. tuberculosis* by the immune system of the infected host. Therefore, differences in the PGRS sequences could represent the principal source of antigenic variation in the otherwise genetically and antigenically homogeneous bacterium.

By performing the method of the invention using the *M. tuberculosis* BAC based DNA library I-1945, the inventors have discovered the occurrence of sequence differences between a given PGRS encoding ORF (ORF reference on the genomic sequence of *M. tuberculosis* Rv0746) of *M. tuberculosis* and its counterpart sequence in the genome of *M. bovis* BCG.

More precisely, the inventors have determined that one ORF contained in BAC vector N° Rv418 of the *M. tuberculosis* BCG I-1945 DNA library carries both base additions and base deletions when compared with the corresponding ORF in the genome of *M. bovis* BCG that is contained in the BAC vector N° X0175 of the *M. bovis* BCG I-XXXX DNA library. The variations observed in the base sequences correspond to variations in the C-terminal part of the aminoacid sequence of the PGRS ORF translation product.

As shown in Figure 6, an amino acid stretch of 9 residues in length is present in this *M. tuberculosis* PGRS (ORF reference Rv0746) and is absent from the ORF counterpart of *M. bovis* BCG, namely the following amino acid sequence:

NH₂-GGAGGAGGSSAGGGGAGGAGGAGGWLLGD-COOH.

Furthermore, Figure 6 shows also that an amino acid stretch of 45 residues in length is absent from this *M. tuberculosis* PGRS and is present in the ORF counterpart of *M. bovis* BCG, namely following amino acid sequence:

NH₂-GAGGIGGIGGNANGGAGGNGGTGGQLWGSGGAGVEGGAAL
SVGDT-COOH.

Similar observations were made with PPE ORF Rv0442, which showed a 5 codon deletion relative to a *M. bovis* amino acid sequence.

Given that the polymorphism associated with the PE-PGRS or PEE ORFS resulted in extensive antigenic variability or reduced antigen presentation, this would be of immense significance for vaccine design, for understanding

protective immunity in tuberculosis and, possibly, explain the varied responses seen in different BCG vaccination programmes.

There are several striking parallels between the PGRS proteins and the Epstein-Barr virus-encoded nuclear antigens (EBNA). Both polypeptide families are glycine-rich, contain Gly-Ala repeats that represent more than one third of the molecule, and display variation in the length of the repeat region between different isolates. The Gly-Ala repeat region of EBNA1 has been shown to function as a *cis*-acting inhibitor of antigen processing and MHC class I-restricted antigen presentation (Levitskaya et al., 1995). The fact that MHC class I knock-out mice are extremely susceptible to *M. tuberculosis* underlines the importance of MHC class I antigen presentation in protection against tuberculosis. Therefore, it is possible that the PE/PPE protein family also play some role in inhibiting antigen presentation, allowing the bacillus to hide from the host's immune system.

As such the novel and nonobvious PGRS polynucleotide from *M. bovis* which is homolog to the *M. tuberculosis* ORF Rv0746, and which is contained in the BAC clone N° X0175 (See Table 4 for SP6 and T7 end-sequences of clone n° X0175) of the I-XXXX *M. bovis* BCG BAC DNA library is part of the present invention, as it represents a starting material in order to define specific probes or primers useful for detection of antigenic variability in mycobacterial strains, possible inhibition of antigen processing as well as to differentiate *M. tuberculosis* from *M. bovis* BCG.

Thus, a further object of the invention consists in a polynucleotide comprising the sequence SEQ ID N°4.

Polynucleotides of interest have been defined by the inventors as useful detection tools in order to differentiate *M. tuberculosis* from *M. bovis* BCG. Such polynucleotides are contained in the 45 aminoacid length coding sequence that is present in *M. bovis* BCG but absent from *M. tuberculosis*. This polynucleotide has a sequence beginning (5'end) at the nucleotide at position nt 729 of the sequence SEQ ID N°4 and ending (3'end) at the nucleotide in position nt 863 of the sequence SEQ ID N°4.

Thus, part of the present invention is also a polynucleotide which is chosen among the following group of polynucleotides :

a) a polynucleotide comprising at least 8 consecutive nucleotides of the nucleotide sequence SEQ ID N°5 ;

Rv106; Rv39; Rv255; Rv74; Rv355; Rv268; Rv58; Rv173; Rv264; Rv417; Rv401; Rv144; Rv302; Rv81; Rv163; Rv281; Rv221; Rv420; Rv175; Rv86; Rv412; Rv73; Rv269; Rv214; Rv287; Rv42; Rv143.

5 The polynucleotides disclosed in Table 3 may be used as probes in order to select a given clone of the BAC DNA library I-1945 for further use.

The invention also provides for a BAC-based *Mycobacterium bovis* strain Pasteur genomic DNA library that has been deposited in the Collection Nationale de Cultures de Microorganismes on XXXX XX, 1998 under the accession number I-XXXX.

10 A further object of the invention consists in a recombinant BAC vector which is chosen among the group consisting of the recombinant BAC vectors belonging to the BAC-based DNA library I-XXXX. This DNA library contains approximately 1600 clones. The average insert size is estimated to be ~80 kb.

Generally, a recombinant BAC vector of interest may be chosen among
15 the following set or group of BAC vectors contained in the BAC-based DNA library I-XXXX :

X0001; X0002; X0003; X0004; X0006; X0007; X0008; X0009; X0010; X0012; X0013; X0014; X0015; X0016; X0017; X0018; X0019; X0020; X0021; X0175.

20 The end sequences of the polynucleotide inserts of each of the above clones corresponding respectively to the sequences adjacent to the T7 promoter and to the Sp6 promoter on the BAC vector are shown in Table 4.

The polynucleotides disclosed in Table 4 may be used as probes in order to select a given clone of the BAC DNA library I-XXXX for further use.

25 Are also part of the invention the polynucleotide inserts that are contained in the above described BAC vectors, that are useful as primers or probes.

These polynucleotides and nucleic acid fragments may be used as primers for use in amplification reactions, or as nucleic acid probes.

30 PCR is described in the US patent N° 4,683,202. The amplified fragments may be identified by an agarose or a polyacrylamide gel electrophoresis, or by a capillary electrophoresis or alternatively by a chromatography technique (gel filtration, hydrophobic chromatography or ion exchange chromatography). The specificity of the amplification may be ensured by a molecular hybridization using, for example, one of the initial primers as nucleic acid probes.

35 Amplified nucleotide fragments are used as probes in hybridization reactions in order to detect the presence of one polynucleotide according to the

Table 4 : End-sequences of the polynucleotide inserts cloned in the named recombinant BAC vectors contained in the I-XXXX *M. bovis* strain Pasteur genomic DNA library.

RvXXXSP6 corresponds to the SP6 end-sequence of the clone RvXXX.

RvXXXT7 corresponds to the T7 end-sequence of the clone RvXXX.

RvXXXIS 1081 corresponds to a region located close to a copy of the IS1081 repetitive sequence (Insertion element).

The character « - » denotes an uncertain base residue.

Clone X0001

.....:X0001SP6.seq:.....

AAG-

TCGGGTTTCCACACGCGCGGTTTGACCCTAGTCATATGTAATCATGTGTACCATGTGCGGGCGCTTTTCGACGGCCG
CGAACCACCGGA-ATTTCTGTGATTTCACTGCATGCGTACCATCTGGCACAATTGAGCA-TTGTCT-
TCGCGGTGGTCGG-CGGGTTGCGTGCCGCCTGCTGCGA-ATGCACCA-
TAAGCCCGAACCACCGGCTTGGTGACCACCGCACGCTGCGTGTGGGGGTAACCACTCCGCGACCCCAAGGATGGT
CATTTCCAATGAACGGGCTGGACTTCGTCCA-A

.....:X0001T7.seq:.....

GTCGCGGTTTCGATCGACCCGATCTTCACCTCGTAACCTCGATGCTTAGCAGGATCCAGCTTGACCGCGTTTGGCTCT
ACCACTCTTTGAGTGGCGCCGTCGCCTGTGCCCCATCGGTGTTTCATGACGAACGCTTCGAAAGACTTCCTCTTGTG
AGCCGGAATGTCTGCGTAAAGAAGTTCCATGTCCGGGAAGTAGACCCGGTGCCTCCACGTGGTACTCCTTCGAGG
TCCGCTTCTCGCCGATCCGATAAACACCGGCCCCAGGCACCGCAGCGTGAGTTCGAACGGCTTCAGGTAGGTGTTT
ATGCGGCGGACTCCGGGAGTGCGAGAAATAGCGGTGCGCGTAGCTGTAGACCGGATGGTTTCCGCCAGGCTGACG
TCGAAGATGCCTCCTTGGAAGGGGCGCGA

Clone X0002

.....:X0002SP6.seq:.....

AACTCAAGTTTTACGGTGATCGCGCATCACCTGGTTCATGAACTGGAAGCAGCGCAGCGCTTCCTTTTCGGCCGCA
ACATGAGCCAGCCTCTCGTCGGCGGTTCGGGTGCAAGTGCTCGGGCAGCTCGGCCGCGACAGCCGCCTGACCCTGAAA
CCAGCTTCCATATCCC GCGAC-
AACGACGCCAGTCCGCTACGTAACCCCTCCGCGACTGTCCATGGACAACAGCGCGTTCCTCCACCGACCGGGCCCGG
TGT

.....:X0002T7.seq:.....

GTGCAGGTTTCGACAATGTGGTGCCGGTTCGGCGGCTACGTGCCATCGAGACACTGGCGCA-GCTATCGCACCCGTT
ATCGGCTGCGAGCAAATCGCGGTATGCGTTCTTGAGCATGAGTCGGCGACCGTCGTTCATGGTCGACACCCACGACGG
AAAGACGCAGATCGCCGTCGAAGCATGTGTGCCGCGGATTATCAGGACTGACCTCCTGGCTGACCGGCATGTTTGGTC
GCGATGCCTGGCGCCCGGCGGTGGTCGTGGTCGGCTCGGATAGCGAGGTCAGCGAATTCCTGTGGCAGCTCGAA
AGGTCCTGCCGGTGCCGCT

Clone X0003

.....:X0003SP6.seq:.....

TTTCGAGTCATGCGCCCGCCTCGACCACGAA-ATGCACGTCG-
GGTTCGATCGACCCGATCTTCACCTCGTAACCTCGATGCTTAGCAGGATCCAGCTTGACCGCGTTTGGCTCTACCCA
CTCTTTGAGTGGCGCCGTCGCTGTGCCCCATCGGTGTTTCATGACGAACGCTTCGAAAGACTTCCTCTTGTGAGCCG
GAATGCTCTGCGTAAAGAAGTTCCATGTCCGGGAAGTAGACCCGGTGCCTCCACGTGGTACTCCTTCGAGGTCCGC
TTCTC

CLAIMS

1. A method for isolating a polynucleotide of interest that is present in a genome of a first mycobacterium strain or that is expressed by said first mycobacterium strain and that is absent or altered in a genome of a second mycobacterium strain that is different from the first mycobacterium strain or that is not expressed in the second mycobacterium strain, said method comprising:
- 5 a) contacting under hybridizing conditions the genomic DNA of the first mycobacterium strain with the DNA of at least one clone that belongs to a bacterial artificial chromosome (BAC) genomic DNA library of the second mycobacterium strain ; and
- 10 b) isolating the polynucleotide of interest that fails to form a hybrid with the DNA of the second mycobacterium strain.
2. The method according to claim 1, wherein the BAC-based DNA library
- 15 has been constructed from genomic DNA of *Mycobacterium tuberculosis*.
3. The method according to claim 2, wherein the BAC-based DNA library has been constructed from genomic DNA of *Mycobacterium tuberculosis* strain H37Rv.
4. The method according to claim 3, wherein the BAC-based DNA library
- 20 has been deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) on November 19, 1997 under the accession number I-1945.
5. The method according to claim 1, wherein the BAC-based DNA library has been constructed from genomic DNA of *Mycobacterium bovis*.
6. The method according to claim 5, wherein the BAC-based DNA library
- 25 has been constructed from the genomic DNA of *Mycobacterium bovis* BCG strain Pasteur.
7. The method according to claim 6, wherein the at least one BAC-based DNA library has been deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) on XX XX, 1998 under the accession number I-
- 30 XXXX.
8. A method of isolating a polynucleotide of interest that is present in a genome of a first mycobacterium strain or that is expressed by the first mycobacterium strain and that is absent or altered in a genome of a second mycobacterium strain or that is not expressed by the second mycobacterium strain, said method comprising :
- 35

- a) providing at least one polynucleotide contained in a clone of a bacterial artificial chromosome (BAC) DNA library of the first mycobacterium strain;
- b) providing at least one genomic or cDNA polynucleotide from a second mycobacterium strain that is different from the first mycobacterium strain or at
- 5 least one polynucleotide contained in a clone of a BAC DNA library prepared from the genome of the second mycobacterium strain;
- c) contacting under hybridizing conditions the polynucleotide of step a) with the polynucleotide of step b); and
- d) isolating the polynucleotide of step a) that has not formed a hybrid complex
- 10 with the polynucleotide of step b).

9. The method of claim 8, wherein the polynucleotide contained in a clone of a BAC DNA library of the first or second mycobacterium strain is prepared by the following procedure :

- 1) digesting at least one recombinant BAC clone by an appropriate restriction
- 15 endonuclease to yield a polynucleotide insert of interest; and
- 2) isolating the polynucleotide insert of interest.

10. A purified polynucleotide of interest that has been isolated according to the method of claim 8.

11. The purified polynucleotide of claim 10 which contains at least one
- 20 Open Reading Frame (ORF).

12. The purified polynucleotide of claim 11, which is SEQ ID N0:1.

13. The purified polynucleotide of claim 11, wherein said polynucleotide is selected from the group consisting of :

- a) a polynucleotide comprising at least 8 consecutive nucleotides of SEQ ID
- 25 N0:1 ;
- b) a polynucleotide having a sequence fully complementary to SEQ ID N°:1 ; and
- c) a polynucleotide that hybridizes under stringent hybridization conditions with the polynucleotide defined in a) or with the polynucleotide defined in b).

14. The purified polynucleotide of claim 13, which is SEQ ID N0:2.

15. The purified polynucleotide of claim 13, which is SEQ ID N0:3.

16. The purified polynucleotide of claim 11, wherein the ORF encodes all or part of a polypeptide involved in the pathogenicity of a mycobacterium strain.

17. The purified polynucleotide of claim 11, wherein the ORF encodes all or part of a Polymorphism Glycine Rich Sequence (PGRS).

18. The purified polynucleotide of claim 17, which is SEQ ID N0:4.

19. The purified polynucleotide of claim 17, which is selected from the group consisting of :

a) a polynucleotide comprising at least 8 consecutive nucleotides the of SEQ ID N0:5 ;

b) a polynucleotide having a sequence that is fully complementary to SEQ ID N0:5 ;

c) a polynucleotide that hybridizes under stringent hybridization conditions with the polynucleotide defined in a) or with the polynucleotide defined in b).

20. A pair of the purified polynucleotides as claimed in claim 10.

21. A *Mycobacterium tuberculosis* strain Rv37 genomic DNA library that has been deposited in the Collection Nationale de Cultures de Microorganismes under accession number I-1945, wherein said genomic DNA library comprises recombinant bacterial artificial chromosome vectors.

22. A recombinant bacterial artificial chromosome (BAC) vector, which belongs to the genomic DNA library of claim 21.

23. The recombinant BAC vector of claim 22, which is selected from the group consisting of :

Rv101; Rv102; Rv103; Rv104; Rv105; Rv106; Rv107; Rv108; Rv109; Rv10;
Rv110; Rv111; Rv112; Rv113; Rv114; Rv115; Rv116; Rv117; Rv118; Rv119;
Rv11; Rv120; Rv121; Rv122; Rv123; Rv124; Rv126; Rv127; Rv128; Rv129;
Rv130; Rv132; Rv134; Rv135; Rv136; Rv137; Rv138; Rv139; Rv13; Rv140;
Rv141; Rv142; Rv143; Rv144; Rv145; Rv146; Rv147; Rv148; Rv149; Rv14;
Rv150; Rv151; Rv152; Rv153; Rv154; Rv155; Rv156; Rv157; Rv159; Rv15;
Rv160; Rv161; Rv162; Rv163; Rv164; Rv165; Rv166; Rv167; Rv169; Rv16;
Rv170; Rv171; Rv172; Rv173; Rv174; Rv175; Rv176; Rv177; Rv178; Rv179;
Rv17; Rv180; Rv181; Rv182; Rv183; Rv184; Rv185; Rv186; Rv187; Rv188;
Rv18; Rv190; Rv191; Rv192; Rv193; Rv194; Rv195; Rv196; Rv19; Rv1; Rv201;
Rv204; Rv205; Rv207; Rv209; Rv20; Rv214; Rv215; Rv217; Rv218; Rv219;
Rv21; Rv220; Rv221; Rv222; Rv223; Rv224; Rv225; Rv226; Rv227; Rv228;
Rv229; Rv22; Rv230; Rv231; Rv232; Rv233; Rv234; Rv235; Rv237; Rv240;
Rv241; Rv243; Rv244; Rv245; Rv246; Rv247; Rv249; Rv24; Rv251; Rv252;
Rv253; Rv254; Rv255; Rv257; Rv258; Rv259; Rv25; Rv260; Rv261; Rv262;
Rv263; Rv264; Rv265; Rv266; Rv267; Rv268; Rv269; Rv26; Rv270; Rv271;
Rv272; Rv273; Rv274; Rv275; Rv276; Rv277; Rv278; Rv279; Rv27; Rv280;

Rv281; Rv282; Rv283; Rv284; Rv285; Rv286; Rv287; Rv288; Rv289; Rv28;
 Rv290; Rv291; Rv292; Rv293; Rv294; Rv295; Rv296; Rv29; Rv2; Rv301;
 Rv302; Rv303; Rv304; Rv306; Rv307; Rv308; Rv309; Rv30; Rv310; Rv311;
 Rv312; Rv313; Rv314; Rv315; Rv316; Rv317; Rv318; Rv319; Rv31; Rv32;
 5 Rv322; Rv327; Rv328; Rv329; Rv32; Rv330; Rv331; Rv333; Rv334; Rv335;
 Rv336; Rv337; Rv338; Rv339; Rv33; Rv340; Rv341; Rv343; Rv344; Rv346;
 Rv347; Rv348; Rv349; Rv34; Rv350; Rv351; Rv352; Rv353; Rv354; Rv355;
 Rv356; Rv357; Rv358; Rv359; Rv35; Rv360; Rv361; Rv363; Rv364; Rv365;
 Rv366; Rv367; Rv368; Rv369; Rv36; Rv370; Rv371; Rv373; Rv374; Rv375;
 10 Rv376; Rv377; Rv378; Rv379; Rv37; Rv381; Rv382; Rv383; Rv384; Rv385;
 Rv386; Rv387; Rv388; Rv389; Rv38; Rv390; Rv391; Rv392; Rv393; Rv396;
 Rv39; Rv3; Rv40; Rv412; Rv413; Rv414; Rv415; Rv416; Rv417; Rv418; Rv419;
 Rv41; Rv42; Rv43; Rv44; Rv45; Rv46; Rv47; Rv48; Rv49; Rv4; Rv50; Rv51;
 Rv52; Rv53; Rv54; Rv55; Rv56; Rv57; Rv58; Rv59; Rv5; Rv60; Rv61; Rv62;
 15 Rv63; Rv64; Rv65; Rv66; Rv67; Rv68; Rv69; Rv6; Rv70; Rv71; Rv72; Rv73;
 Rv74; Rv75; Rv76; Rv77; Rv78; Rv79; Rv7; Rv80; Rv81; Rv82; Rv83; Rv84;
 Rv85; Rv86; Rv87; Rv88; Rv89; Rv8; Rv90; Rv91; Rv92; Rv94; Rv95; Rv96
 and Rv9.

24. The recombinant BAC vector of claim 22, which is selected from the
 20 group consisting of :
 Rv234; Rv351; Rv166; Rv35; Rv415; Rv404; Rv209; Rv272; Rv30; Rv228;
 Rv233; Rv38; Rv280; Rv177; Rv48; Rv374; Rv151; Rv238; Rv156; Rv92; Rv3;
 Rv403; Rv322; Rv243; Rv330; Rv285; Rv233; Rv219; Rv416; Rv67; Rv222;
 Rv149; Rv279; Rv87; Rv273; Rv266; Rv25; Rv136; Rv414; Rv13; Rv289; Rv60;
 25 Rv104; Rv5; Rv165; Rv215; Rv329; Rv240; Rv19; Rv74; Rv411; Rv167; Rv56;
 Rv80; Rv164; Rv59; Rv313; Rv265; Rv308; Rv220; Rv258; Rv339; Rv121;
 Rv419; Rv418; Rv45; Rv217; Rv134; Rv17; Rv103; Rv21; Rv22; Rv2; Rv270;
 Rv267; Rv174; Rv257; Rv44; Rv71; Rv7; Rv27; Rv191; Rv230; Rv128; Rv407;
 Rv106; Rv39; Rv255; Rv74; Rv355; Rv268; Rv58; Rv173; Rv264; Rv417;
 30 Rv401; Rv144; Rv302; Rv81; Rv163; Rv281; Rv221; Rv420; Rv175; Rv86;
 Rv412; Rv73; Rv269; Rv214; Rv287; Rv42 and Rv143.

25. A *Mycobacterium bovis* BCG strain Pasteur genomic DNA library,
 wherein said genomic DNA library comprises recombinant bacterial artificial
 chromosome vectors.

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26. A recombinant bacterial artificial chromosome (BAC) vector, which belongs to the genomic DNA library of claim 25.

27. A recombinant BAC vector according to claim 26, which is selected from the group consisting of :

5 X0001; X0002; X0003; X0004; X0006; X0007; X0008; X0009; X0010; X0012; X0013; X0014; X0015; X0016; X0017; X0018; X0019; X0020; X0021 and X0175.

28. A method for detecting a mycobacterial nucleic acid in a biological sample comprising the steps of :

10 a) contacting the recombinant BAC vector according to claim 22 or 26, or a purified polynucleotide according to claim 10 with the mycobacterial nucleic acid in the biological sample ; and
b) detecting a hybrid nucleic acid molecule formed between said recombinant BAC vector or said purified polynucleotide and the mycobacterial nucleic acid in
15 the biological sample.

29. The method of claim 28, further comprising before step a), making the mycobacterial nucleic acid in the biological sample available to a hybridization reaction.

20 30. A method for detecting mycobacterial nucleic acid in a biological sample comprising the steps of :

a) contacting a first polynucleotide according to claim 10 that has been immobilized onto a substrate with the mycobacterial nucleic acid in the biological sample ; and
b) contacting a hybrid nucleic acid molecule formed between said first
25 polynucleotide and the mycobacterial nucleic acid in the biological sample with a second, labeled polynucleotide according to claim 10, wherein said second polynucleotide and said first polynucleotide have non-overlapping sequences.

31. The method of claim 30, further comprising before step a), making the mycobacterial nucleic acid in the biological sample available to a hybridization
30 reaction.

32. The method of claim 30 or 31, further comprising before step b), removing the mycobacterial nucleic acid that is not hybridized with the immobilized first polynucleotide.

33. A method for detecting mycobacterial nucleic acid in a biological
35 sample comprising the steps of :

- a) contacting the mycobacterial nucleic acid in the biological sample with a pair of purified polynucleotides according to claim 20 ;
- b) amplifying said mycobacterial nucleic acid ; and
- c) detecting the amplified mycobacterial nucleic acid.

5 34. The method of claim 33, further comprising before step a), making the mycobacterial nucleic acid in the biological sample available to a hybridization reaction.

 35. A kit for detecting a mycobacterium in a biological sample comprising :

- 10 a) a recombinant BAC vector according to claim 22 or 26, or a purified polynucleotide according to claim 10 ; and
- b) reagents necessary to perform a nucleic acid hybridization reaction.

 36. A kit for detecting a mycobacterium in a biological sample comprising :

- 15 a) a recombinant BAC vector according to claim 22 or 26, or a first polynucleotide according to claim 10 that is immobilized onto a substrate ;
- b) reagents necessary to perform a nucleic acid hybridization reaction ; and
- c) a second polynucleotide according to claim 10, wherein said second polynucleotide is radioactively or non-radioactively labeled, and wherein said
- 20 second polynucleotide and said first polynucleotide have non-overlapping sequences.

 37. A kit for detecting a mycobacterium in a biological sample comprising :

- a) a pair of purified polynucleotides according to claim 20 ; and
- 25 b) reagents necessary to perform a nucleic acid amplification reaction.

 38. A method for detecting the presence of a genomic DNA, a cDNA or a mRNA of a mycobacterium in a biological sample, comprising the steps of :

- a) contacting the biological sample with a plurality of BAC vectors according to claim 22 or 26, or purified polynucleotides according to claim 10 that are
- 30 immobilized on a substrate ; and
- b) detecting the hybrid complexes formed.

 39. A kit for detecting a genomic DNA, a cDNA or a mRNA of a mycobacterium in a biological sample, comprising :

- a) a substrate on which a plurality of BAC vectors according to claim 22 or 26, or
- 35 purified polynucleotides according to claim 10 have been immobilized.

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40. A method for detecting a polynucleotide of mycobacterial origin in a biological sample, said method comprising :

- a) aligning at least one polynucleotide contained in a recombinant BAC vector according to claim 22 or 26 on the surface of a substrate ;
- 5 b) contacting the polynucleotide in the biological sample with the substrate on which the polynucleotide of step a) has been aligned ; and
- c) detecting a hybrid nucleic acid molecule formed between the polynucleotide in the biological sample and the aligned polynucleotide of step a).

41. A kit for detecting a polynucleotide of mycobacterial origin in a biological sample, comprising :

- 10 a) a substrate on which at least one polynucleotide contained in a recombinant BAC vector according to claim 22 or 26 has been aligned.

42. The method of claim 9, wherein the procedure by which the polynucleotide contained in a clone of a BAC DNA library is prepared, further
15 comprises amplifying the polynucleotide insert.

43. The method of claim 9, wherein the procedure by which the polynucleotide contained in a clone of a BAC DNA library is prepared, further comprises digesting the polynucleotide insert with at least one restriction endonuclease.

20 44. The method of claim 42, further comprising digesting the amplified polynucleotide insert with at least one restriction endonuclease.

45. The Polynucleotide of claim 16, wherein the mycobacterium strain is *Mycobacterium tuberculosis*.

25 46. The method of claim 33, wherein the amplified mycobacterial DNA is detected by gel electrophoresis or with a labeled polynucleotide according to claim 10.

47. The kit of claim 37, further comprising a polynucleotide according to claim 10.

30 48. The kit of claim 39, further comprising reagents necessary to perform a hybridization reaction.

49. A method for physically mapping a polynucleotide of mycobacterial origin in a biological sample, said method comprising:

- a) aligning at least one polynucleotide contained in a recombinant BAC vector according to claim 22 or 26 on the surface of a substrate;

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- b) contacting the polynucleotide in the biological sample with the substrate on which the polynucleotide of step a) has been aligned under hybridizing conditions; and
- c) detecting the location of the hybridized polynucleotide from the biological sample.
- 5

50. The kit of claim 41, further comprising reagents necessary for labeling DNA and reagents necessary for performing a hybridization reaction.